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Carbohydrate Research 307 (1998) 253–262

Oxidation of threose-series pentoses and hexoses by sodium *N*-chloro-*p*-toluenesulfonamide

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Received 17 November 1997; accepted 25 January 1998

Abstract

The kinetics and mechanism of oxidation of threose-series hexoses and pentoses by chloramine-T in alkaline medium was investigated. Kinetic studies with D-galactose, D-sorbose, D-xylose, and D-lyxose showed that the rate of the reaction was first order with respect to sugar and chloramine-T, and second order with respect to hydroxide ion. *p*-Toluenesulfonamide and chloride ions, the reduced products of chloramine-T, have no effect on the reaction rate. The rate increases with increase in ionic strength of the medium, and the dielectric effect is negative. Proton inventory studies in H_2O–D_2O mixtures suggested a single transition state. Product analysis for D-gulose, D-idose, L-sorbose, D-galactose, D-talose, D-tagatose, D-xylose, and D-lyxose revealed that all lyxose-series hexoses gave mainly mixtures of lyxonic and threonic acids with minor proportions of hexonic, xylonic and glyceric acids, whereas all xylose-series hexoses gave mixtures of lyxonic, threonic and glyceric acids with minor amounts of xylonic and hexonic acids. Xylose and lyxose gave mixtures consisting mainly of lyxonic, threonic, and glyceric acids with minor proportions of xylonic acid. From the results of kinetic studies, reaction stoichiometry, and product analysis, a possible mechanism for the oxidation of threose-series sugars with chloramine-T is suggested.

Keywords: Threose-series sugars; Oxidation with chloramine-T; Kinetics and mechanism

1. Introduction

Recently, we reported on the kinetics and mechanism of oxidation of erythrose-series sugars, D-glucose, D-mannose, D-fructose, D-arabinose and D-ribose, with chloramine-T (CAT) in alkaline medium at 35 °C [1]. The observed reaction stoichiometry, 2–3 moles of CAT per mole of sugar, was significantly different from the previously reported sugar to oxidant stoichiometry of 1:1 for aldoses and 1:2 for fructose [2–4]. We have also shown by HPLC and GLC-MS analyses that the products of oxidation for erythrose-series sugars (both pentoses and hexoses) were mixtures of aldonic acids consisting of arabinonic, ribonic, erythronic, and glyceric acids [1]. These product

Abbreviations: CAT, RNClNa or chloramine-T, sodium salt of *N*-chloro-*p*-toluenesulfonamide; S, sugar, D. dielectric, *T*, absolute temperature; E_a, activation energy; *n*, atom fraction of deuterium, TS, transition state; RS, reactant site; *I*, ionic strength; d_{AB}, size of the activated complex; *k*, Boltzmann constant; HPLC, high-performance liquid chromatography; GLC-MS, gas–liquid chromatography–mass spectrometry.

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0008-6215/98/$19.00
PII S0008-6215(98)00046-9

profiles were also different from those reported previously, the corresponding aldonic acids for aldoses and arabinonic acid for fructose [2–4].

Our recent study on the oxidation of erythrose-series sugars with CAT gave two interesting results [1]. First, the sugars that can exist in the furanose ring form in appreciable proportions reacted with CAT much faster than those which exist almost exclusively in the pyranose form. Thus, for hexoses, rate of oxidation of fructose was higher than glucose and mannose. Similarly, ribose was oxidized faster than arabinose. Second, surprisingly the products formed from both pentoses and hexoses, including keto-hexoses, were strikingly similar for all erythrose-series sugars studied. Based on these results, we proposed a novel pathway for the oxidation of erythrose-series sugars by CAT [1]. In the present study, the mechanism of oxidation of threose-series sugars by CAT was investigated by kinetic studies and product analysis. The results demonstrate that the kinetic and thermodynamic properties, and mechanism of oxidation of threose-sieres sugars are generally similar to those observed for the erythrose-series sugars.

2. Experimental

Materials.—Chloramine-T (E. Merck) was purified from dichloro contaminants by washing with CCl_4. D-galactose, D-xylose, D-lyxose, D-idose, D-talose, D-tagatose, D-gulose, D-gluconic acid, D-galactono-1,4-lactone, and D-ribono-1,4-lactone, were purchased from Sigma Chemical Co. (St. Louis, MO). L-sorbose, D-mannono-1,4-lactone, D-xylono-1,4-lactone, and D-arabinono-1,4-lactone, were from Pfanstiehl (Waukegan, IL). D_2O (99.4%) was from Bhabha Atomic Research Center (Bombay, India).

Kinetic measurements.—The reactions were carried out in glass stoppered pyrex boiling tubes coated black on the outside [1]. Pseudo-first order conditions ($[sugar]_0 >> [CAT]_0$) were maintained for all kinetic studies. Stock solutions of alkali, CAT, sugars, and $NaClO_4$ were maintained at 35 °C. From these stock solutions, the requisite mixtures of sugars, alkali, and $NaClO_4$ were prepared. The reaction was initiated by the addition of CAT and monitored by iodometric determination of unconsumed CAT at various time-intervals. Sodium perchlorate was used to "swamp" the reaction. The solvent isotopic studies were performed with D_2O.

Pseudo-first order rate constants (k_{obs}) were calculated from the plots of $\log[CAT]_0$ versus time, and these were within ±3%. Regression coefficient r and the standard deviation s, were determined by regression analysis of the experimental data using an EC-72 statistical calculator.

Stoichiometry and product analysis.—The reaction mixtures containing sugar (0.01 M), alkali (0.1 M), and CAT (0.05 M) were kept for 24 h at 35 °C. The unconsumed CAT was determined iodometrically. From these data, the amount of the oxidant consumed per mole of sugar was calculated.

The oxidation products were analyzed by a Dionex HPLC system with pulsed amperometric detection using a CarboPac PA1 high-pH anion-exchange column (4×250 mm) as reported previously [1,5]. Isocratic elution with 0.2 M NaOH was used. The products were identified by comparison of the retention times with retention times of the standard aldonic acids, as reported previously [1].

3. Results

The kinetics of oxidation of threose-series sugars with CAT were generally similar to those observed previously for the erythrose-series sugars [1]. When sugars were used in excess, the plots of log[CAT] versus time were linear ($r > 0.9980$, $s \leq 0.02$), indicating a first-order dependence of reaction rate on $[CAT]_0$. The pseudo-first-order rate constants (k_{obs}) calculated from these plots are shown in Table 1. The rate increased with increase in $[S]_0$ (where S = sugar) and the plots of $\log k_{obs}$ versus $\log[S]_0$ were linear ($r > 0.9998$, $s \leq 0.01$) with unit slopes, indicating a first-order dependence with respect to sugars. Furthermore, the plots of k_{obs} versus $[S]_0$ passed through the origin ($r > 0.9855$, $s \leq 0.04$), suggesting that the sugar–oxidant complexes have only transient existence.

The rate of oxidation also increased with an increase in alkali concentration (Table 2). The plots of $\log k_{obs}$ versus $\log[HO^-]$ ($r > 0.9990$, $s \leq 0.01$) indicated that the reactions follow second-order dependence on $[HO^-]$.

Addition of *p*-toluenesulfonamide (0 to 0.008 M) did not affect the reaction rate, suggesting that the reduced product of CAT, *p*-toluenesulfonamide, is

Table 1
Effect of reactant concentrations on the rate of oxidation of sugars by CAT at 35 °C

10^3 $[CAT]_0$ (m)	10^2 $[S]_0$ (m)	10^4 $k_{obs}(s^{-1})$			
		D-galactose	L-sorbose	D-xylose	D-lyxose
1.5	2.0	3.2	13.3	14.0	5.8
2.0	2.0	3.0	12.9	13.4	5.5
2.5	2.0	3.1	12.9	13.4	5.6
3.0	2.0	3.2	13.0	13.9	5.6
3.5	2.0	3.2	13.0	14.1	5.5
4.0	2.0	3.3	13.0	14.4	5.6
2.0	0.6	0.9	3.8	4.9	1.6
2.0	0.8	1.2	5.0	6.0	2.1
2.0	1.0	1.5	6.3	7.8	2.7
2.0	3.0	4.9	19.8	23.8	8.4
2.0	4.0	6.6	26.3	33.3	11.3
2.0	6.0	10.2	41.9	64.0	15.4

$[HO^-] = 0.1$ M, and $[I] = 0.4$ M.

not involved in pre-equilibrium with the oxidant. Addition of NaCl (0 to 0.02 M) to the reaction mixtures had no effect on the rates, suggesting that the free chloride ion was not formed before the rate-limiting step.

Addition of $NaClO_4$ (0 to 0.8 M) increased the rate of reaction. The plots of log k_{obs} versus $I^{1/2}$ (I = the ionic strength of medium) were linear with fractional slopes of 0.60–0.85 (not shown).

The rate decreased with an increase in methanol content. The plots of log k_{obs} versus 1/D ($r > 0.9970$, $s \leq 0.04$; D = dielectric constant of the medium) were linear with negative slopes (Fig. 1).

The Arrhenius plots of log k_{obs} versus $1/T$ (T = absolute temperature), for reactions studied over a range of temperatures (303 to 318 K), were found to be linear ($r > 0.9991$, $s \leq 0.01$) (not shown). The activation energies (E_a, Table 3) were calculated from the slopes of the plots. From the values of E_a and the thermodynamic parameters $\Delta H^{\#}$, $\Delta S^{\#}$, and $\Delta G^{\#}$ (Table 3) were computed. Sorbose and xylose have lower E_a and $\Delta S^{\#}$ values as compared with other sugars studied. A low enthalpy change for sorbose and a negative enthalpy change for xylose suggest that the structures of sorbose and xylose are favorably disposed for oxidation by CAT. Thus, a keto-enolic anion appears to be the readily reacting structure for hexoses and an aldo-enolic one for pentoses. Previously, it was observed, for erythrose-series sugars, that a fructose-enolic anion is the readily reacting structure for the hexoses and an aldo-enolic form is that reacting for the pentoses [1].

Table 2
Effect of [NaOH] on the rate of oxidation of sugars by CAT at 35 °C

10^2 [NaOH] (m)	10^4 $k_{obs}(s^{-1})$			
	D-galactose	L-sorbose	D-xylose	D-lyxose
2.0	0.2	0.5	1.1	0.3
4.0	0.6	2.1	3.4	1.0
6.0	1.5	5.0	7.1	2.4
8.0	2.2	8.0	11.3	4.1
10.0	3.0	12.9	13.4	5.5
15.0	6.8	36.2	38.4	17.8

$[CAT]_0 = 0.002$ M, $[S]_0 = 0.02$ M, and $[I] = 0.4$ M.

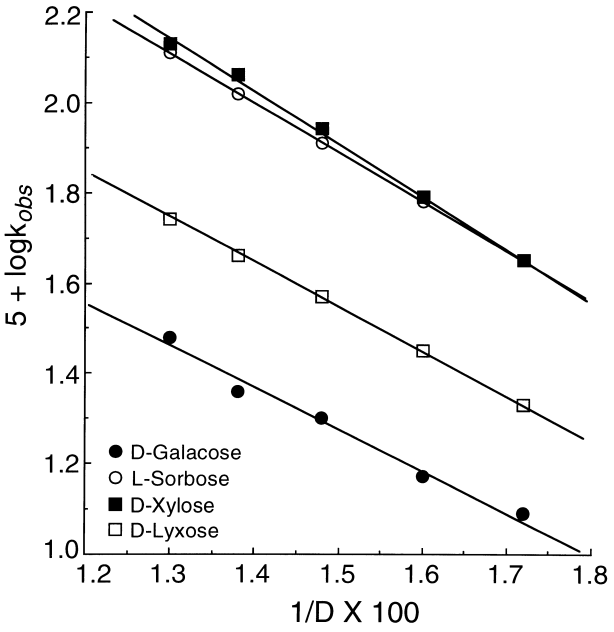


Fig. 1. Plots of logk_{obs} versus 1/D. $[CAT]_0 = 0.002$ M, $[S]_0 = 0.002$ M, $[OH^-] = 0.1$ M, $[I] = 0.4$ M; temperature = 35 °C.

The oxidation of sugars by CAT was studied in H_2O–D_2O mixtures containing varying deuterium atom fractions n. As in the case of erythrose-series sugars [1], the oxidation was faster in D_2O for the threose-series sugars (Table 4). The solvent-isotope effects, $k_{obs}(H_2O)/k_{obs}(D_2O)$, were between 0.5 and

Table 3
Thermodynamic parameters for the oxidation of sugars by CAT at 35 °C

Sugars	E_a (kJ mol^{-1})	$\Delta H^{\#}$ (kJ mol^{-1})	$\Delta G^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$ (JK^{-1} mol^{-1})	Log A
D-Galactose	121	119	96	72	21
L-Sorbose	98	96	93	9	17
D-Xylose	91	89	93	−12	16
D-Lyxose	111	109	95	44	19

$[CAT]_0 = 0.002$ M, $[HO^-] = 0.1$ M, $[S]_0 = 0.05$ M, and $[I] = 0.4$ M.

Table 4
Proton inventory studies for the oxidation of sugars by CAT in H_2O–D_2O mixtures at 35 °C

Atom fraction of deuterium (n)	$10^4\ k_{obs}(s^{-1})$			
	D-galactose	L-sorbose	D-xylose	D-lyxose
0.00	3.2	12.9	13.4	5.5
0.25	3.4	15.4	16.1	6.4
0.50	4.0	18.3	18.6	7.6
0.75	5.0	22.0	22.3	8.9
0.93	5.8	25.5	25.9	10.4

$[CAT]_0 = 0.002$ M, $[HO^-] = 0.1$ M, $[S]_0 = 0.02$ M, and $[I] = 0.4$ M.

0.6 for all sugars (Table 4). Proton-inventory plots (not shown), $k_{obs}(H_2O)/k_{obs}(D_2O)$ versus n , were linear and similar to those obtained previously for erythrose-series sugars (1).

The oxidant to sugar stoichiometry of nearly 3 was observed for all sugars except for lyxose (Table 5).

Although kinetic studies could not be carried out for gulose, idose, talose and tagatose due to the limited availability of the sugars, the oxidation products were analyzed for all threose-series hexoses (Fig. 2). HPLC analysis indicated that lyxonic, xylonic, threonic, and glyceric acids are the products of oxidation for all threose-series hexoses and pentoses (Fig. 2a–c, and Table 5). Xylose and lyxose gave major proportions of lyxonic, threonic and glyceric acids and minor proportions of xylonic acid (Fig. 2c). The xylose-series hexoses (gulose, idose, and sorbose) gave predominantly threonic and glyceric, acids with minor proportions of xylonic and lyxonic acids (Fig. 2b). On the other hand, all lyxose-series hexoses (galactose, talose, and tagatose) gave threonic and lyxonic acids as predominant products with small amounts of glyceric and xylonic acids (Fig. 2a). All hexoses except sorbose gave minor amounts of hexonic acids (Fig. 2a and b). Furthermore, all threose-series sugars except lyxose and galactose were oxidized almost quantitatively by CAT; after 24 h incubation with CAT at 35 °C, 20–25% of lyxose and 5% of galactose remained unoxidized (Fig. 2a–c).

The oxidation products were analyzed at 0.5, 1, 2, 4, 8, 20, and 24 h for all the sugars. The relative proportions of various aldonic acids formed were similar at all time-points analyzed. Approximately 95% of sorbose and idose were oxidized by CAT in 4 h at 35 °C, whereas 90% oxidation of gulose required about 8 h. When gulose and idose were treated with alkali in the absence of CAT, a gradual build up of sorbose was observed for both sugars; for example, about 30% of gulose was isomerized to sorbose in 2 h. However, when gulose and idose were treated with both CAT and alkali, only a minor proportion of sorbose was detected from each reaction mixture at all time-points analyzed, suggesting that sorbose formed by the alkali-catalyzed isomerization readily reacts with CAT. Sorbose treated with alkali alone was not isomerized to gulose and idose to detectable levels. These data indicate that the keto-isomer is the reactive species.

About 95% of tagatose was oxidized by CAT in 2 h at 35 °C, whereas oxidation of similar amounts of galactose and talose required ~20 h. When galactose and talose were treated with alkali alone, significant amounts of tagatose formed by the isomerization of these sugars could be detected. For example, 17% of talose and 23% of galactose were

Table 5
HPLC analysis of the products formed by the oxidation of sugars by CAT in alkaline medium

Sugar	Mol of CAT consumed per mol of sugar	Products (approximate percentage) [a]				
		Glyceric acid	Threonic acid, erythronic acid [b]	Xylonic acid	Lyxonic acid	Hexonic acid
D-Galactose	2.8	9	44	3	32	12
D-Talose	n.d.	9	45	2	32	11
D-Tagatose	n.d.	12	47	2	27	12
D-Gulose	2.9	38	38	12	5	7
D-Idose	n.d.	40	40	8	5	7
L-Sorbose	n.d.	44	44	9	3	—
D-Xylose	2.8	20	37	7	36	—
D-Lyxose	1.9	25	32	6	37	—

[a] Based on the peak areas.
[b] Peak 2 in Fig. 2 represents 95–96% threonic acid and 4–5% threonic acid, which can be separated by using a low flow rate.
n.d., Not determined.

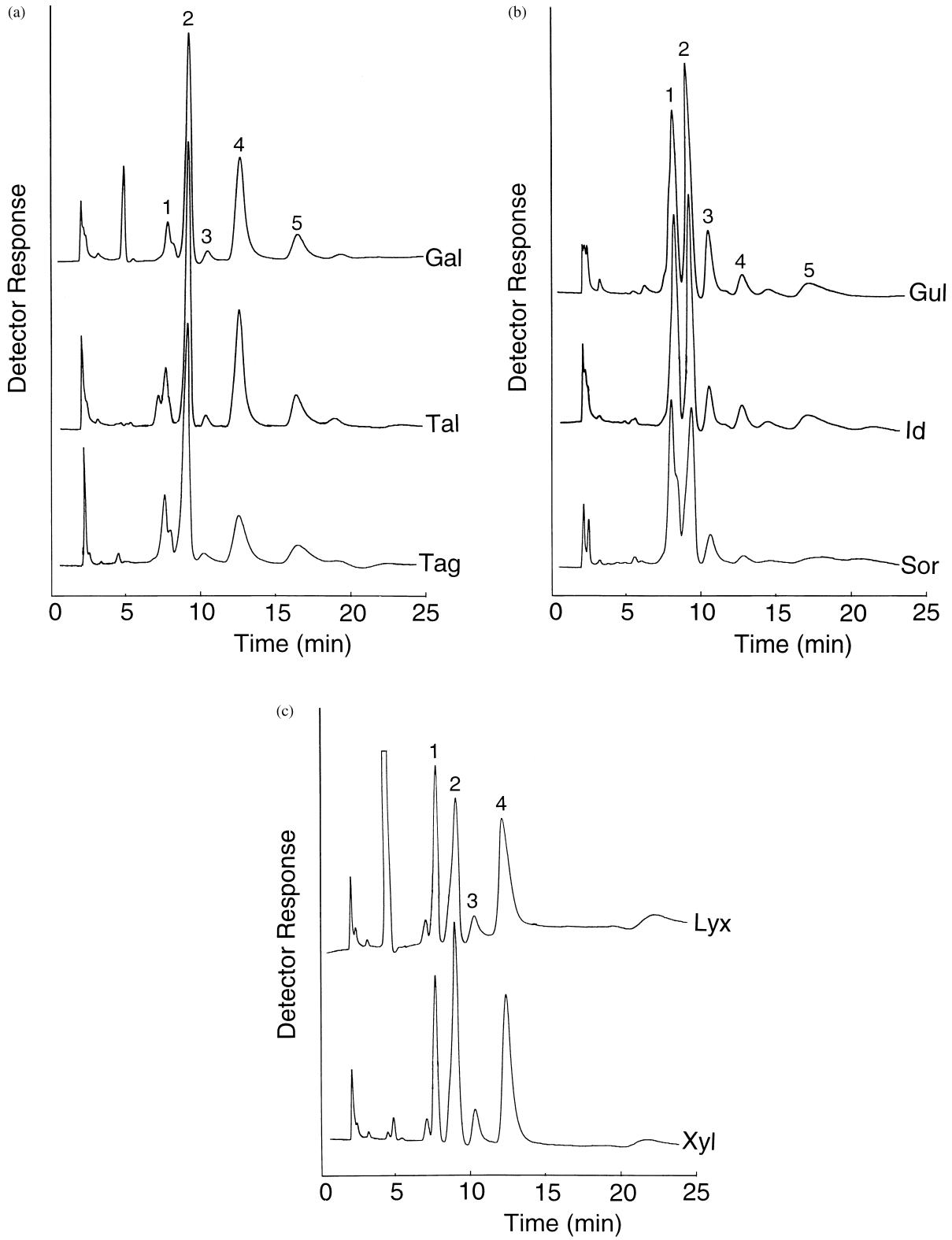


Fig. 2. HPLC analysis of the products formed by the oxidation of sugars (0.01 M) by CAT (0.05 M) in the presence of alkali (0.1 M) at 35 °C. 1, glyceric acid; 2, threonic acid (~95%) plus erythronic acid (~5%); 3, xylonic acid; 4, lyxonic acid; 5, hexonic acids. Gal, Tal, Tag, Gul, Ido, Sor, Xyl and Lyx, respectively represent HPLC chromatograms of the reaction of CAT with D-galactose, D-talose, D-tagatose, D-gulose, D-idose, L-sorbose, D-xylose, and D-lyxose. Peak 2 in (a)–(c) represents 95–96% threonic acid and 4–5% threonic acid which can be separated by using a low flow rate. Note: about 20–25% of lyxose (retention time 4.6 min in (c)) and ~5% galactose (retention time 5.0 min in (a)), and 1–2% of xylose (retention time 5.0 min in (c) were not oxidized by CAT; all other sugars were almost quantitatively oxidized.

isomerized to tagatose in 4 h. However, when talose was treated with both CAT and alkali, unoxidized talose was accounted by 1% tagatose, 1% galactose, and 98% talose. Similarly, when galactose was treated with both CAT and alkali, tagatose was barely detectable. Upon treatment with alkali at 35 °C, only 1–2% of tagatose was isomerized into galactose and talose. Together, these data indicated that for lyxose-series hexoses, as in the case of the xylo-series hexoses, the keto-isomer (tagatose) is the reactive species.

All of the aldohexoses studied here were oxidized mainly to pentonic and tetronic acids rather than to hexonic acids. This finding is in agreement with the foregoing conclusion that hexoses are oxidized by CAT in the keto-enolic form. Moreover, we have previously shown, based on the ease of aldose–ketose isomerization, and relative rates of oxidation and product profiles, that glucose and mannose undergo oxidation by CAT predominantly in the fructose-enolic form [1].

For all the hexoses studied here, threonic and pentonic acids were formed within 30 min; hexonic acids were detectable in minor proportions only after significant amounts of the former products are formed. This demonstrate that the lower-carbon aldonic acids were not derived from the initially formed six-carbon aldonic acids. We have previously shown that D-gluconic, D-mannonic, D-galactonic, D-ribonic, and D-arabinonic acids were not oxidized by CAT [1].

Formation of high proportions of pentonic acids from xylose and lyxose suggest that pentoses react with CAT predominantly in the aldo-enolic form. The formation of glyceric acid also in high proportion suggest that appreciable amounts of xylose and lyxose react in the keto-enolic form to give glyceric and threonic acids.

4. Discussion

In aqueous solutions, sodium salts of aryl-*N*-halosulfonamides ionize into several species in a pH dependent manner [6–8]. In acidic solutions, the oxidizing species of CAT are RNClH, $RNCl_2$, and HOCl, while in alkaline solution $RNCl^-$ is the active oxidant [6–10].

In alkaline solutions, sugars undergo isomerization to an equilibrium mixture of aldoses and ketoses which exist as enediol anions (E^-) [11]. The enolic-anions (E^-, keto-enolic anions of hexoses and aldo-enolic anions of pentoses) react with $RNCl^-$ to form an intermediate (**X**), which undergoes cleavage to form products (Fig. 3). From the results of the kinetic study, the oxidation of sugars is predicted to proceed through the reaction sequences shown in Scheme 1.

Under steady state conditions for E^-, the rate of

$$S + HO^- \underset{k_{-1}}{\overset{k_1 \text{ slow}}{\rightleftharpoons}} E^- + H_2O \quad \text{(i)}$$

$$E^- + HO^- + RNCl^- \xrightarrow[\text{(Slow and rate determining step)}]{k_2} X \quad \text{(ii)}$$

$$X \xrightarrow[\text{Fast}]{k_3} \text{Products} \quad \text{(iii)}$$

Scheme 1.

disappearance of CAT is given by

$$\text{Rate} = -\frac{\text{d[CAT]}}{\text{d}t} = \frac{k_2k_1[\text{S}][\text{HO}^-]^2[\text{CAT}]}{k_{-1}[\text{H}_2)] + k_2[\text{HO}^{-1}][\text{CAT}]} \quad (1)$$

Since $k_{-1}[H_2O] > k_2[HO^-][CAT]$, rate law (1) is reduced to

$$\text{Rate} = -\frac{\text{d[CAT]}}{\text{dt}} = \frac{k_2k_1[\text{S}][\text{HO}^-]^2[\text{CAT}]}{k_{-1}[\text{H}_2\text{O}]} \quad (2)$$

which agrees with the observed rate $= k_{obs}[S][OH^-]^2[CAT]$.

The observed first-order dependence of rate on $[CAT]_0$ and $[S]_0$ and second-order dependence on $[HO^-]$ agree with eq (2).

Since DO^- is a stronger base than HO^- by a factor of 2, the reaction rate is expected to be doubled in D_2O for reactions involving a fast pre-equilibrium H^+ or HO^- ion transfer [12]. In agreement with this, the observed values of the inverse solvent isotope effect $k_{obs}(D_2O)/k_{obs}(H_2O)$ were approximately 2 for the sugars (Table 4). The results of proton inventory plots (not shown) are also in accordance with Scheme 1. The dependence of rate constant (k^n_{obs}) on n (n = the atom fraction of deuterium in a solvent mixture of H_2O and D_2O) [13,14] is given by the Gross–Butler eq (3).

$$k^n_{obs}/k^o_{obs} = \frac{\prod^{TS}(1-n+n\phi_i)}{\prod^{RS}(1-n+n\phi_j)} \quad (3)$$

where ϕ_i and ϕ_j are isotopic fractionation factors for the isotopically exchangeable hydrogen sites in the transition state (TS) and reactant site (RS), respectively. If the reaction proceeds through a single transition state, then eq (3) becomes eq (4):

$$\left(k^n_{obs}/k_{obs}\right) = 1 + n(\phi_j - 1) \quad (4)$$

A comparison of the plots of k_{obs} versus n (not shown) with the standard curves [15], suggested a single proton exchange in the transition state. Furthermore, the plots of k^n_{obs}/k_{obs} versus n (Fig. 1) were linear with slopes $(\phi_j - 1)$. The ϕ_j for the oxidation of sugars by CAT is about 0.6. This value resembles the fractionation factor of HO^- ion.

Since the rate-determining step in Scheme 1 involves three negative ions, the reaction rate is expected to increase with an increase in the ionic strength (I) of reaction medium. The plots of logk_{obs} versus $I^{1/2}$ were linear with slopes between 0.5 and 0.85, even though the ionic strengths employed were beyond the Debye–Huckel range. The theoretical slope of unity has not been realized, possibly due to the formation of Bjerrum ion pairs in concentrated solutions [16].

The rate decreased with decrease in the dielectric constant (D) of the reaction medium. The plots of logk_{obs} versus 1/D (D = dielectric constant) were linear with negative slopes (Fig. 1). The effect of solvent composition on the rate of a reaction involving two negative ions is given by eq (5) [16]:

$$\log k = \log k_o - Z_A Z_B e^2/DkTd_{AB} \quad (5)$$

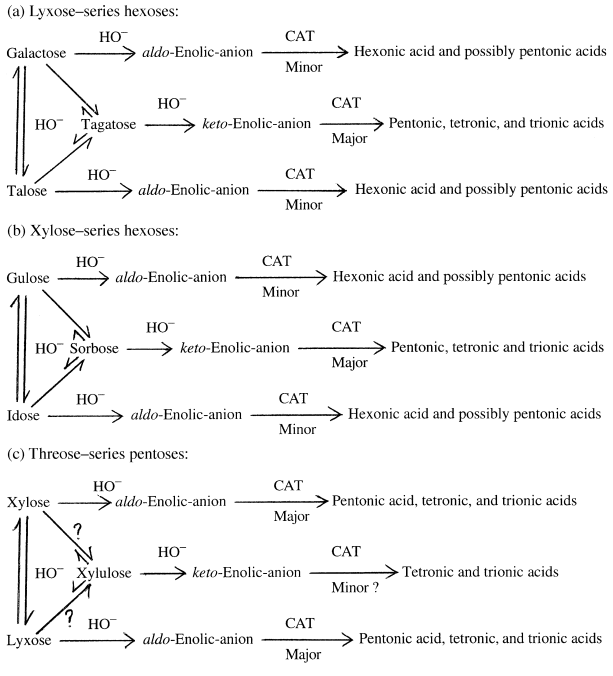
where k_o is the rate constant in a medium of infinite dielectric constant, $Z_A e$, and $Z_B e$ are the charges on ions, d_{AB} is the size of the activated complex, k is the Boltzmann constant, and T is the absolute temperature. From the slopes of the straight lines in Fig. 1 (slope $= -Z_A Z_B e^2/kT, d_{AB}$), d_{AB} was calculated. The derived d_{AB} values for D-galactose, L-sorbose, D-xylose, and D-lyxose are 2.70, 2.20, 2.02, and 2.40 Å, respectively. These values are comparable with those obtained for similar reactions [16].

According to Scheme 1, the rate-determining step should involve interactions between similarly charged ions (eq (ii) requiring a very high activation energy. The observed high activation energies (Table 3) agree with this prediction. Nearly constant $\Delta G^{\#}$ values (Table 3) suggest that a common mechanism is operative for the oxidation of sugars.

In a previous study on erythrose-series hexoses [1], it was observed that each of the sugars studied was oxidized by CAT predominantly to pentonic and threonic acids. However, for the threose-series hexoses studied here, the product profiles can be grouped into three categories, profiles consisting of: (a) predominant proportions of lyxonic and threonic acid formed from lyxose-series sugars (Fig. 2a), (b) threonic and glyceric acids as major products formed from xylose-series hexoses (Fig. 2b), and (c) high proportions of pentonic, threonic, and glyceric acids formed from xylose and lyxose (Fig. 2c). Based on these results and on the observed isomerization of sugars in alkaline solutions, the following reaction pathway (Scheme 2) can be suggested:

The observed reaction stoichiometry (Table 5) agrees with the formation of mixtures of pentonic, tetronic and trionic acids.

For lyxose-series hexoses, the major products are formed by the loss of one or two carbon atoms with the cleavage of C-1–C-2 and C-2–C-3 bonds, respectively from the keto-enolic anion intermediates. The predominance of lyxonic acid and



Scheme 2.

minor amounts of xylonic acid from all lyxose-series sugars suggest that, for these sugars, the cleavage of the C-1–C-2 bond occurs without appreciable epimerization at C-3 (Fig. 2a). The formation of significant amounts of hexonic acid from these sugars, suggest that appreciable proportions of the sugars react with CAT in the aldo-enolic forms, which up on cleavage of the C-1–H bond form hexonic acids. Minor amounts of pentonic acids are formed by the cleavage of the C-1–C-2 bond from the aldo-enolic anions of hexoses. The formation of hexonic acid from tagatose is presumably due to isomerization to the aldo-enolic form.

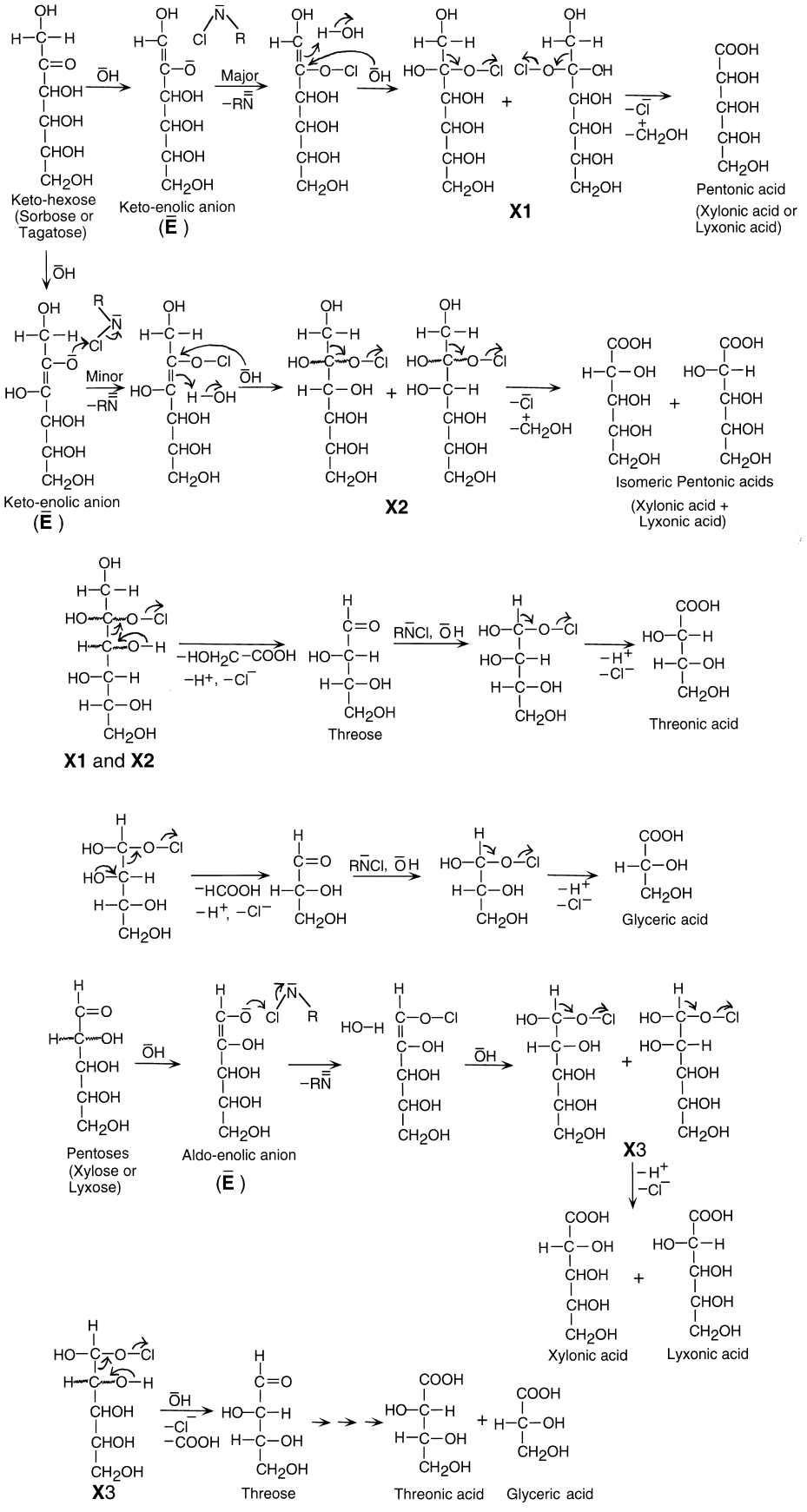
The xylose-series hexoses, gulose, idose and sorbose, gave mainly threonic and glyceric acids and minor proportions of pentonic acids (xylonic and negligible amounts of lyxonic acid) (Fig. 2b). The predominance of threonic and glyceric acids indicate the preferential cleavage of C-2–C-3 and C-3–C-4 bonds compared with C-1–C-2 bonds. Since xylonic acid was formed in higher proportion compared with lyxonic acid, the cleavage of the C-1–C-2 bond in xylose-series hexoses must occur without appreciable epimerization at C-3, as in the case of lyxose-series hexoses (cf. Fig. 2b with Fig. 2a). Formation of small amounts of hexonic acids from gulose and idose was presumably due to slow oxidation of the aldo-enolic form. Formation of only a trace amount of hexonic acid from sorbose is due to negligible isomerization of sorbose to gulose and idose.

In contrast to hexoses, xylose and lyxose gave high proportions of pentonic acids (Fig. 2c). Clearly, these major products are formed by the cleavage of the C-1–H bond. Both xylose and lyxose also gave high proportions of threonic and glyceric acids, which are formed by cleavage of the C-1–C-2 and C-2–C-3 bonds, respectively. Since the latter type of bond-cleavage is facilitated through the involvement of the keto-enolic form, it is possible that portions of pentoses react in the keto-enolic form. However, lyxose and xylose were not isomerized to xylulose to a significant level. Together, these data suggest that pentoses undergo oxidation by CAT mainly through aldo-enolic intermediates, and only minor proportions may be oxidized via the keto-enolic form. In accordance with the observed three-times faster reaction rate of xylose compared with lyxose, a large amount of lyxose was not oxidized by CAT, even after 24 h at 35 °C (Fig. 2c). Under similar conditions, xylose was almost completely oxidized.

In view of the foregoing considerations, a plausible mechanism for the oxidation of sugars by CAT is proposed in Scheme 3. This mechanism accounts for the observed kinetics, reaction stoichiometry, and products formed.

In the proposed mechanism (Scheme 3), the anions (E^-) of sugars react with CAT to form intermediates **X1**–**X3**. For threose-series hexoses, the anions (E^-) intermediates are predominantly the keto-enolic forms and minor proportions of aldo-enolic forms. However, for pentoses, the major reacting species are the aldo-enolic anions; probably minor proportions of keto-isomer may also be involved. In the case of anions (E^-) from hexoses, the loss of hydrogen can occur at either C-1 or C-3 to form C-1–C-2 or C-2–C-3 enediols containing a hypochlorite group at C-2. Since epimerization at C-3 was limited, as evidenced by the formation of only very minor proportions of epimeric pentonic acids from hexoses, it can be concluded that cleavage of the C-1–H bond occurs preferentially as compared with cleavage of the C-3–H bond to form C-1–C-2 enediols. The ene-diols thus formed contain polarized double bonds to which hydroxide ion can add at C-2 to form intermediates **X1** (major) and **X2** (minor). **X1** and **X2** then can undergo cleavage of C–C bonds between C-1 and C-2, the former giving lyxonic acid and the latter forming a mixture of lyxonic and xylonic acids.

In the case of aldo-enolic anions from pentoses, hydrogen can be removed only from C-2 to form the C-1–C-2 enediol-anion, which in the presence of CAT and alkali forms intermediate **X3** with epimerization at C-2. The cleavage of C-1–H bonds from **X3** gives a mixture of lyxonic and xylonic acids. The cleavage of C–C bonds between C-2 and C-3 in **X1** and **X2**, and the breaking of C–C bonds between C-1 and C-2 in **X3** yield aldo-tetrose without epimerization at C-4 (hexoses) or at C-3 (pentoses). The aldo-tetrose further oxidizes to yield threonic acid and a minor proportion of erythronic acid (Table 5). The reaction can proceed further, with the cleavage of C–C bonds between C-3 and C-4 of hexoses and the breaking of C–C bonds between C-2 and C-3 of pentoses, to form glyceric acid. Minor proportions of threonic and glyceric acids could also be formed by the cleavage of C-1–C-2 and C-2–C-3 bonds, respectively, from the keto-enolic form of pentoses through the reactions sequences similar to those outlined for keto-hexoses in Scheme 3.


Keto-hexose
(Sorbose or
Tagatose)
Keto-enolic anion
(Ē)
Major
−RN̄
X1
Pentonic acid
(Xylonic acid or
Lyxonic acid)
Minor
−RN̄
Keto-enolic anion
(Ē)
X2
Isomeric Pentonic acids
(Xylonic acid +
Lyxonic acid)
X1 and X2
Threose
Threonic acid
Glyceric acid
Pentoses
(Xylose or
Lyxose)
Aldo-enolic anion
(Ē)
X3
Xylonic acid
Lyxonic acid
X3
Threose
Threonic acid
Glyceric acid

Scheme 3.

Acknowledgements

The proposed mechanism for the reaction is based on suggestions given by Professor Derek Horton for the oxidation of erythrose-series sugars by CAT [1], and we thank Professor Horton for his help. H.M and M.P.R. are grateful to the University of Mysore for the University Grants Commission, India, research fellowships. K.S.R. thanks the CSIR, India, for the financial support.

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